

Patent Application No.: 09/580,797

Attorney Docket No.: UNMC63149

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (canceled)
2. (previously amended) A method of determining whether one or more fungal *Aspergillus* species is present in a sample of fungi, said method comprising the following steps:
  - a) extracting nucleic acid material from fungi contained in a patient sample from a patient suspected of having an *Aspergillus* infection;
  - b) adding two oligonucleotide primers, one of said primers consisting of SEQ ID NO:1 and the other primer consisting of SEQ ID NO:2, said primers bracketing a hypervariable region on the rRNA present in the fungal species of said group, and said primers being capable of amplifying *Aspergillus ustus* (SEQ ID NO: 3), *Aspergillus terreus* (SEQ ID NO: 4), *Aspergillus niger* (SEQ ID NO: 5), *Aspergillus nidulans* (SEQ ID NO: 6), *Aspergillus fumigatus* (SEQ ID NO: 7), and *Aspergillus flavus* (SEQ ID NO: 8);
  - c) amplifying the sequence between said primers; and
  - d) using one or more detectably labeled probes directed to a portion of the hypervariable region bracketed by said primers, said probes being selected from the group consisting of at least 15-25 contiguous nucleotides of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8 which distinguish said species, each said labeled probe being specific for one of said fungal species from said group, to determine whether said fungal species identified by each said labeled probe is present in said sample.

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3. (previously amended) The method of claim 2, wherein said amplifying procedure is the polymerase chain reaction.

4. (previously amended) The method of claim 2 in which said one or more probes hybridize to a nucleic acid sequence encoding the internal spacer regions of a pathogenic *Aspergillus* species gene sequence and is selected from the group consisting of (SEQ ID NO:3), (SEQ ID NO:4), (SEQ ID NO:5), (SEQ ID NO:6), (SEQ ID NO:7), and (SEQ ID NO:8).

5. (previously amended) The method of claim 2 wherein, in step (d), more than one probe is used, each said probe being connected to (a) a different signal moiety or (b) a moiety which allows separation of said probes.

6-19. (canceled)

20. (currently amended) A method for determining which *Aspergillus* species selected from the group consisting of *Aspergillus ustus* (SEQ ID NO: 3), *Aspergillus terreus* (SEQ ID NO: 4), *Aspergillus niger* (SEQ ID NO: 5), *Aspergillus nidulans* (SEQ ID NO: 6), *Aspergillus fumigatus* (SEQ ID NO: 7), and *Aspergillus flavus* (SEQ ID NO: 8) is present in a biological sample, said method comprising comparing the sequences of fungal nucleic acid extracted from said biological sample with the nucleic acid sequences of SEQ ID NOs: 3-8 to determine which pathogenic *Aspergillus* species is present in said biological sample, each of said sequences of SEQ ID NOs: 3-8 being amplified by polymerase chain reaction using a primer set consisting of SEQ ID NOs: 1 and 2.

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21. (currently amended) A method for determining which *Aspergillus* species is present in a biological sample, said species being selected from the group consisting of *Aspergillus ustus* (SEQ ID NO: 3), *Aspergillus terreus* (SEQ ID NO: 4), *Aspergillus niger* (SEQ ID NO: 5), *Aspergillus nidulans* (SEQ ID NO: 6), *Aspergillus fumigatus* (SEQ ID NO: 7), and *Aspergillus flavus* (SEQ ID NO: 8), wherein each of said sequences of SEQ ID NOs: 3-8 is amplifiable with polymerase chain reaction using a primer set consisting of SEQ ID NOs: 1 and 2, said method comprising the steps of:

- a) extracting fungal nucleic acid from said biological sample;
- b) generating restriction mapping patterns of said fungal nucleic acid; and
- c) comparing said restriction mapping patterns of said fungal nucleic acid to the restriction mapping patterns of the nucleic acid sequences of SEQ ID NOs: 3-8, wherein identical restriction mapping patterns are indicative of which *Aspergillus* species is present in said biological sample.

22. (previously presented) A method for determining which *Aspergillus* species selected from the group consisting of *Aspergillus ustus* (SEQ ID NO: 3), *Aspergillus terreus* (SEQ ID NO: 4), *Aspergillus niger* (SEQ ID NO: 5), *Aspergillus nidulans* (SEQ ID NO: 6), *Aspergillus fumigatus* (SEQ ID NO: 7), and *Aspergillus flavus* (SEQ ID NO: 8) is present in a biological sample, said method comprising the steps of:

- a) obtaining permeabilized tissue sections containing fungal nucleic acid from a patient;
- b) contacting said permeabilized tissue sections with fluorescent molecular probes specific for pathogenic *Aspergillus* species comprising the sequences of SEQ ID NOs: 3-8; and

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c) analyzing said permeabilized tissue section for said fluorescent molecular probes, the detection of which is indicative of the presence of pathogenic *Aspergillus* species in said biological sample.

23. (new) A universal primer set for amplification of a target DNA sequence associated with pathogenic strains of fungi, said primer set consisting of the following sequences:

GGAAGTAAAAGTCGTAACAAGG (SEQ ID NO: 1) and

GTATCCCTACCTGATCCGAGG (SEQ ID NO: 2).

24. (new) A kit for identifying pathogenic fungal species in a biological sample, said kit comprising:

a) a universal primer set, said primer set consisting of the sequence of SEQ ID NO: 1 and SEQ ID NO: 2;

b) lysis buffer suitable for lysing fungus in said biological sample, such that DNA is released from said fungus upon exposure to said buffer;

c) a polymerase enzyme suitable for use in polymerase chain reaction;

d) means for contacting said released DNA with a primer set consisting of the sequence of SEQ ID NO: 1 and NO: 2 under conditions where amplification of pathogenicity-associated ITS sequences occurs, if said pathogenic fungus is present in said sample; and

e) means for detecting said amplified sequence, if present.

25. (new) A kit as claimed in claim 24, wherein said amplified sequence is detected via incorporation of a detectable label.

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26. (new) A kit as claimed in claim 24, wherein said amplified sequence is detected by gel electrophoresis of said amplified sample.

27. (new) A kit as claimed in claim 24, wherein said amplified sequence is compared to a sequence selected from the group consisting of SEQ ID NOs: 3-33, thereby identifying said pathogenic fungus if present.